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APPLICATION NUMBER: ***60/041,921***
FILING DATE: ***April 7, 1997***

PRIORITY DOCUMENT

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. R. Grant

P. R. GRANT
Certifying Officer

Our Ref.: 7841-057

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Assistant Commissioner for Patents
BOX PROVISIONAL
Washington, D.C.
U.S.A. 20231

Dear Sir:

PROVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(b)(2)

This is a request for filing a Provisional application under 37 C.F.R. § 1.53(b)(2) entitled **Intravenous Immune Globulin Formulation Containing A Non-Ionic Surface Active Agent With Improved Pharmacokinetic Properties** by the following inventors:

Full Name of Inventor	**Family Name** Price	**First Given Name** Hugh	**Second Given Name**
Residence & Citizenship	**City** Winnipeg	**State or Foreign Country** Manitoba	**Country of Citizenship**
Post Office Address	**Post Office Address** c/o Cangene Corporation 104 Chancellor Matheson Road	**City** Winnipeg	**State & Zip Code/Country** Manitoba, Canada R3T 2N2
Full Name of Inventor	**Family Name** Woloski	**First Given Name** Michael	**Second Given Name** R.
Residence & Citizenship	**City** Winnipeg	**State or Foreign Country** Manitoba	**Country of Citizenship**
Post Office Address	**Post Office Address** c/o Cangene Corporation 104 Chancellor Matheson Road	**City** Winnipeg	**State & Zip Code/Country** Manitoba, Canada R3T 2N2

1. __√__ Enclosed is the Provisional application as follows: __26__ pages of specification, __12__ claims, __1__ page Abstract and __1__ sheet of drawings.

2. ____ Enclosed is a Verified Statement that this filing is by a small entity (37 C.F.R. 1.9, 1.27, 1.28).

3. __√__ Payment of Provisional filing fee under 37 C.F.R. § 1.16(k):

   ____ Attached is a cheque in the amount of $_____.
   __√__ Please charge the filing fee of $150.00 from our Deposit Account No. 02-2095. The letter is being filed in triplicate.
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4. __√__ The Commissioner is hereby authorized to charge any additional fees as set forth in 37 C.F.R. §§ 1.16 to 1.18 which may be required by this paper or credit any overpayment to Account No. 02-2095.

5. ____ Enclosed is an Assignment of the invention to ______________. Recordation Form Cover Sheet and a cheque for $40 to cover the Recordation Fee.

6. ____ Also enclosed:

7. ____ The invention was made by the following agency of the United States Government or under a contract with the following agency of the United States Government: ______________________________.

8. __√__ Address all future communications to the Attention of Micheline Gravelle at the address below.

Respectfully submitted,

April 4, 1997
Date

M. Gravelle

Micheline Gravelle
Registration No. 40,261

BERESKIN & PARR
Box 401
40 King Street West
Toronto, Ontario
Canada M5H 3Y2
Telephone: 416-364-7311

B&P File No. 7841-057/MG

BERESKIN & PARR **UNITED STATES**

Title: INTRAVENOUS IMMUNE GLOBULIN FORMULATION CONTAINING A NON-IONIC SURFACE ACTIVE AGENT WITH IMPROVED PHARMACOKINETIC PROPERTIES

Inventors: HUGH PRICE and B. MICHAEL R. WOLOSKI

B&P File No. 7841-057

Title: **INTRAVENOUS IMMUNE GLOBULIN FORMULATION CONTAINING A NON-IONIC SURFACE ACTIVE AGENT WITH IMPROVED PHARMACOKINETIC PROPERTIES**

FIELD OF THE INVENTION

The present invention relates to an improved immune globulin formulation containing a non-ionic surface active agent to prolong the serum half-life and to alter the immunomodulatory effect of immune globulin. One aspect of the invention relates to improved formulations of relatively pure and non-aggregated immune globulin suitable for intravenous injection or infusion containing a non-ionic surface active agent. A second aspect of the invention relates to a method of using a non-ionic surface active agent to favourably alter the pharmacokinetics and safety of immune globulin in mammals. Also provided is an improved method of preventing or treating antigen-associated disorders utilizing formulations of the invention.

BACKGROUND OF THE INVENTION

Immune globulins (also known as immunoglobulins or antibodies) are proteins produced by lymphoreticular tissues. There are 6 known classes of immune globulin: IgG, IgM, IgA, IgD, IgE and secretory IgA. IgG (also known as gamma-globulin) is the most abundant and the most therapeutically relevant class of immune globulin. The primary function of immune globulins is to specifically recognize and bind antigens through reversible bonding thereby facilitating the immune system to eliminate the antigens.

IgG is a glycoprotein of approximately 150,000 Daltons consisting 2 "heavy" (gamma) chains and 2 "light" (kappa or gamma) chains held together by disulphide as well as weak covalent bonds. Within the class of IgG, there are 4 subclasses of IgG1, IgG2, IgG3 and IgG4 comprising about 70%, 15%, 10% and 5% of total IgG in normal human serum respectively. These subclasses possess minor antigenic differences among their "heavy" chains resulting in distinct biological actions.

There are principally two types of immune globulins that are available as therapeutic agents: standard immune serum globulin preparations for general use, and immune globulin preparations that recognize specific antigens for use in specific disorders. Commercial examples of products in the former category include numerous brands of intravenous immune globulin (Gamimune N® by Bayer; Sandoglobulin® by Sandoz; Gammar-IV® by Armour) as well as intramuscular immune globulin (Gamastan® by Cutter; Gammar® by Armour). Examples of products in the latter category are hepatitis B immune globulin (H-BIG® by Abbott; Hep-B-Gammagee® by MSD; HyperHep® by Cutter), varicella zoster immune globulin (VZIG by Massachusetts Public Health Biologic Labs.), cytomegalovirus immune globulin (CytoGam® by Connaught) and Rh immune globulin (WinRho® and WinRho SD® by Cangene; HypRho-D® by Miles; Gamulin Rh® by Armour; RhoGAM® by Ortho Diagnostics). The primary therapeutic basis for immune globulins is passive immunity conferred to the recipient through the direct introduction of extraneous "ready-made" antibodies. The major clinical utilities of immune globulins are prophylaxis and/or treatment of antigen-associated disorders.

Immune globulins may be prepared by isolation of natural immune globulins from mammalian serum. Immune globulins prepared using Cohn's cold ethanol fractionation method suffers from relatively low product yield and IgG purity. The resultant product contain significant amount of aggregated immune globulin which combines with complement (also termed anticomplementary activity) and produce adverse reactions in recipients if given by intravenous injection or infusion (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962). Correspondingly, these immune globulin preparations must be injected intramuscularly (therefore termed intramuscular immune globulin). Intramuscular injections are painful. Drug absorption and peak levels of immune globulin (hence onset of

therapeutic action) are slow, and approximately half of the injected dose is lost due to local proteolysis and incomplete absorption. Significant amounts of IgA and IgM are also present in Cohn-prepared intramuscular immune globulin preparations which can cause anaphylactic reactions in certain recipients.

Intravenous injection or infusion of immune globulin is the preferred route of drug administration in the clinical setting due to its instant bioavailability and rapid onset of therapeutic protection as compared to other parenteral routes as reasoned above. Intravenous immune globulin products differ from intramuscular products in two fundamental ways. First, an intravenous preparation must contain a significantly lower amount of aggregated immune globulin molecules (over about 94% monomeric immune globulin) thereby causing less anticomplementary adverse reactions. Second, the IgG content and product purity of intravenous immune globulin products are significantly higher (over about 95% IgG content) than intramuscular products. The low level of contamination with IgA or IgM in intravenous immune globulin (less than about 40 ug/mL) are also associated with lower incidences of adverse events such as anaphylactic reactions especially in agammaglobulinemic recipients.

Improved methods involving further purification of the Cohn immune globulin fractions (see Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969) were consequently developed to render immune globulin produced from cold ethanol fractionation suuitable for intravenous administration. Ultracentrifugation of the immune globulin-containing fraction, or treatment of immune globulin with pepsin, plasmin, a sulfitolytic agent or beta-propriolactone, reduces the anticomplementary activity of the final preparation (see U.S. Patent No. 4,160,763; Barandun, S. et al., *Monogr. Allergy* 9: 39-60, 1975; Stephan, *Vox Sang.* 28: 422-437, 1975; Wells, J.L.V. et al., *Austr. Ann. Med.* 18: 271, 1969; Baumgarten, W. et al., *Vox Sang.* 13: 84, 1967; Merler, E. et al., *Vox Sang.* 13: 102, 1967; Sgouris, J.T. et al., *Vox Sang.* 13: 71, 1967; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962; Nisonoff, A. et al., *Science* 132: 1770-1771,

1960). U.S. Patent No. 3,903,262 describes the reduction of intermolecular disulphide bonds of immune globulin and alkylation of the resultant sulfhydryl groups. Schura of Germany also developed an intravenously injectable immune globulin by adsorption onto hydroxy-ethyl starch. However, these approaches are often technically unfeasible for manufacturing or that the reactants residual in the final preparation have been shown to cause undesirable outcomes such as a reduction in serum half-life of the immune globulin and elicitation of immunogenic reactions in the recipients.

Further improved chromatographic techniques (*e.g.* using DEAE-Sephadex ion-exchange columns in combination with ultrafiltration) were also developed for the manufacture of immune globulins suitable for intravenous injection or infusion from human plasma (see Canadian Patent number 1,168,152; Canadian Patent number 1,201,063; Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964). The use of such chromatographic manufacturing processes also substantially increases product yield to over about 90%.

Immune globulin prepared by the improved processes may be administered by intravenous injection or infusion as well as other parenteral routes. For instance, Cangene's WinRho® and WinRho SD® are produced using a proprietary anion exchange chromatographic process and are the only commercial anti-Rh_oD immune globulin preparations that can be administered safely by intravenous injection or infusion to humans due to their relatively higher IgG purity and monomeric protein content as well as lower IgA/IgM contamination.

Monoclonal immune globulins can be produced using recombinant and hybridoma techniques (see Canadian Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et

al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may also be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody (see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988).

Immune globulin preparations suitable for parenteral injection commonly consist of an immune globulin distributed in a physiologically compatible medium. Said medium may be sterile water for injection (WFI) with or without isotonic amounts of sodium chloride. For example, the recommended diluent for reconstituting commercial intravenous immune globulins such as Iveegam®, Gammagard®, or, Venoglobulin®, is sterile WFI. Sandoglobulin® is supplied with 0.9% (w/v) sodium chloride solution as diluent (see Gahart, B.L. & Nazareno, A.R., Intravenous Medications: a handbook for nurses and allied health professionals, p. 516-521, Mosby, 1997). WinRho SD®, an anti-Rh_oD immune globulin produced by Cangene Corporation, is reconstituted in 0.9% sodium chloride solution for intravenous injection. The immune globulin product by Schura (*supra*) is formulated as a solution of 165 mEq/L sodium ion and 120 mEq/L chloride ion with a final pH of 6.7. The Miles' intravenous immune globulin preparation, Gammimune®, when constituted, has an osmolality of 278 mOsm/L and a pH of 4.0-4.5. U.S. patent Nos. 4,396,608 and 4,499,073 also disclose a low pH (3.5-5.0) and low ionic strength ($\leq$0.001) immune globulin formulation for intravenous injection. The globulin protein concentration in the above preparations ranges from 0.5% to 20%.

Carbohydrates and their derivatives such as glucose, maltose or mannitol, may be included in immune globulin formulations to adjust the tonicity of the preparation. For example, maltose (10%) is included in Miles' intravenous immune globulin preparation,

Gammimune®, to achieve isotonicity. Sucrose (5%) is included in Sandoz's intravenous immune globulin preparation, Sandoglobulin® and Armour's intravenous immune globulin preparation, Gammar-IV®. The commercial intravenous immune globulin preparation, Venoglobulin®, contains 50 mg/mL D-sorbitol. Likewise, amino acids such as glycine or histidine may be added to improve storage stability of the protein. For example, Glycine (0.3M) is included commercial immune globulin preparations such as American Red Cross' intravenous immune globulin preparations, Polygam® and Polygam S/D®; the intramuscular varicella zoster immune globulin preparation by Massachusetts Public Health Biologic Laboratories; and the intramuscular anti-Rh_oD immune globulin preparations by Armour and Miles. U.S. patents Nos. 4,396,608 and 4,597,966 describe the use of glycine and histidine to stabilize immune globulin formulations. European Patent Application No. EP 392,717 describes the use of mannitol and glycine to stabilize and prevent aggregation of immune globulin in formulation. The prolongation of storage shelf-life of immune globulin preparations may also be accomplished by the addition of preservatives including organic mercurial derivatives such as thimerosal.

Surface active agents (also termed surfactants or detergents) are compounds that can lower the surface tension of water. All surface active agents are amphipathic possessing a hydrophobic end (*e.g.* one or more hydrocarbon chain(s)) as well as a hydrophilic moiety (which may or may not be ionic). A surface active agent may be classified as anionic, cationic, or non-ionic type depending on the nature of its hydrophilic moiety. Soaps with carboxylate or sulphonate groups carry net negative charges and are examples of anionic surface active agents. Benzalkonium, an N-benzyl quaternary ammonium chloride and an antibacterial agent, carries a net positive charge and is an example of a cationic surface active agent. A non-ionic surface active agent contains a neutral group such as a carbohydrate which can hydrogen-bond with water.

Tween® and Span® are two types of non-ionic surface active agents. Span® type agents are partial esters of common fatty acids and sugar alcohol anhydrides derived from sorbitol. Tween® type agents are derivatives of Span® products with polyoxyethylene chains attached to non-esterified hydroxyl groups. Their hydrophilic property is associated with the free hydroxyl and/or oxyalkylene groups, and their hydrophobic property is associated with the long chain fatty acids. Examples of commonly used Span® type surface active agents are sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), sorbitan monooleate (Span 80), and sorbitan trioleate (Span 85). A commonly used member of Tween® type surface active agent, Tween 80, is also known as Polysorbate 80, sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivative, polyoxyethylene sorbitan monooleate, polyethylene oxide sorbitan monooleate, sorethytan monooleate, Sorlate, Monitan or Olothorb. Other examples of polyoxyethylene sorbitan surface active agents comprise polyoxyethylene sorbitan monolaurate (Tween 20 or 21), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60 or 61), polyoxyethylene sorbitan tristearate (Tween 65), and polyoxyethylene sorbitan trioleate (Tween 85).

The safety of non-ionic surface active agents in mammals has been studied extensively and established. Previous acute toxicity studies indicated that the LD50 values for Tween® and Span® type non-ionic surface active agents in rats are relatively high at >15 g/kg for oral ingestion and >1.4 g/kg for parenteral injection (*J. Am. Coll. Toxicol.* 3: 1-82, 1984; Varma, R.K. et al., *Arzneimittelforschung* 35: 804-808, 1985; Farkas, W.R. et al., *Pharmacol. Toxicol.* 68:154-156, 1991). Subacute and chronic toxicity studies also showed minimal toxicity after administration of relatively high oral doses to rats (100-200 mg per kg body weight) (Nityanand, S and Kapoor, N.K., *Indian J. Med. Res.* 69: 664-670, 1979). A number of reproductive toxicology studies also did not identify a hazard with the clinical use of non-ionic surface active agents

(Kitchin, K.T. and Ebron, M.D., *Toxicology* 30: 45-47, 1984; Ema, M. et al., *Drug Chem. Toxicol.* 11: 249-260, 1988; Gajdova, M. et al., *Med. Toxicol.* 3: 128-165 and 209-240, 1988).

The inclusion of surface active agents in protein drug preparations has been practised extensively to improved product stability in storage and/or to increase product solubility. Commercially available preparation of granulocyte colony stimulating factor, Neupogen®, contains 0.004% Polysorbate 80 to improve storage stability. Turbersol® is a sterile isotonic solution of Tuberculin in phosphate buffered saline containing 0.0005% Polysorbate 80 as a stabilizer.

With respect to immune globulin preparations, U.S. Patent No. 4,902,500 disclosed an immune globulin formulations with improved storage stability containing at least one polyoxypropylene-polyoxyethylene block polymer (Pluronic 68). PCT Patent Application FR93/00584 published as WO94/16728 describes the inclusion of Polysorbate 80 in a parenteral formulation of an anti-LFA-1 monoclonal antibody for stabilization purposes. U.S. Patent No. 5,215,743 describes the use of Polysorbate 80 to stabilize parenteral formulations of tumour necrosis factor (TNF). These references only disclose the stabilizing effect of non-ionic surface active agents and do not teach their use to favourably alter the immune globulin pharmacokinetics. U.S. Patent No. 5,151,266 teaches a method of treating antibodies with an anionic detergents such as sodium dodecylsulfate (sodium lauryl sulfate), cetyl ammonium sulfate, or taurocholic acid, to increase the solubility of the antibody and to reduce its reticuloendothelial uptake. The claimed method involves the preincubation of an antibody with the anionic detergent and any unreacted anionic detergent is removed before drug administration. The inventors also specified that treatment with anionic detergents did not alter the serum half-life of the antibody.

Current commercial anti-Rh_oD immune globulin preparations containing $\leq$0.01% Polysorbate 80 are RhoGAM® and MICRhoGAM® produced by Ortho Diagnostics Systems Inc. These preparations must be administered only by intramuscular injection due

to relatively high protein aggregation and low product purity (*supra*). There has been no indication, by the manufacturer or otherwise, to suggest that the addition of Polysorbate 80 increases the plasma half-life or alters the immunomodulatory effect of RhoGAM® or MICRhoGAM® and it appears that the inclusion of a non-ionic surface active agent may not alter the pharmacokinetics and immunomodulatory effect of an immune globulin of low purity with high aggregation. Similarly, Polygam® and Polygam S/D® (Red Cross) contains ≤0.01% Polysorbate 80 to improve immune globulin solubility and storage stability. These products contain relatively low IgG content at 90% and there has been no evidence, by the manufacturer or otherwise, to show that Polysorbate 80 increases the plasma half-life or alters the immunomodulatory effect of Polygam® or Polygam S/D®.

With respect to the use of surface active agents in the production of immune globulin, U.S. Patent Nos. 4,371,520 and 4,379,086 describes the use of alkylene oxide polymers such as polyethylene glycol in the fractionation process for isolating immune globulin-rich fractions from plasma. Similarly, U.S. Patent Nos. 4,276,283 and 5,132,406 describe the use of alkylene oxide polymers such as polyethylene glycol in a precipitation step for isolating and purifying immune globulin-rich fractions. These patents do not teach the use of alkylene oxide polymers in immune globulin formulations, and more importantly, the inventors did not examine the effect of alkylene oxide polymers in altering the pharmacokinetics or immunomodulation of immune globulin.

The ability of surface active agents to alter the pharmacologic properties of drugs has been examined to a limited extent, but their usefulness in immune globulin formulations under the clinical setting has not been established. Jekunen, A. et al. (*Acta Oncol.* 35: 267-271, 1996) reported the *in vivo* modulation of antibody kinetics in mice by Polysorbate 80. Intra-tumour administration of the non-ionic surface active agent, Polysorbate 80, improved the targeting of a radiolabeled monoclonal antibody to the tumour and accelerated antibody clearance from the blood. The authors did not indicate in any way that systemic co-

administration of Polysorbate 80 can prolong the persistence of an immune globulin in the circulation of the animal.

Ellis, A.G. et al. (*Cancer Chemother. Pharmacol.* 38: 81-87, 1996) describes the effects of two surface active agents, Cremophor EL and Tween 80, on the pharmacokinetics of a chemotherapeutic non-protein drug, etoposide, in an isolated perfused rat liver experimental model. Co-administration of either surface active agent decreased the elimination half-life of etoposide. Masters, J.R. et al. (*Cancer Chemother. Pharmacol.* 25: 267-273, 1990) decreased the in vivo half-life of the chemotherapeutic drug, thioTEPA in human subjects. Said decreases in plasma half-lives corresponding increase the need for more frequent drug administration to maintain effective plasma drug concentrations and increase the costs associated with therapy in the clinical setting. Moreover, the compounds examined are not proteins, and more specifically, are not immune globulins.

Liu, F. and Liu, D. (*Pharm. Res.* 12: 1060-1064, 1995) demonstrated the ability of Polysorbate 80 to attenuate the clearance of parenterally administered oil-in-water emulsions has been demonstrated. Said oil-in-water emulsions are physicochemically and biochemically different to the immune globulin proteins of the present invention.

The benefits and methods for covalent bonding of amphipathic polymer moieties to proteins are known in the art. For example, the chemical conjugation of polyethylene glycol (PEG) or monomethoxy-polyethylene glycol (MPEG) to a variety of proteins by a variety of different methods has been described (see PCT Patent Application GB94/01844 published as WO 95/06058; U.S. Patent No. 5,349,052; Delgado, C. et al., *Crit. Rev. Ther. Drug Carrier Sys.* 9: 249-304, 1992). One of the major observed advantages of PEG conjugation to a protein is to decrease its rate of clearance from the body and to increase plasma half-life. However, the polymer (MPEG) by itself without conjugation was shown to elicit no effect on the plasma half-life of the proteins. Said amphipathic polymers are distinct from the non-ionic

surface active agents of the present invention. Moreover, the chemical conjugation process requisite for half-life extension and the conjugated proteins are different from the simple co-administration of surface active agent and immune globulin (as separate, unconjugated, entities) in the present invention.

SUMMARY OF THE INVENTION

Immune globulin preparations with novel clinical characteristics and benefits are prepared. The immune globulin preparations of the invention contain an immune globulin with relatively high IgG and low aggregated protein contents and are suitable for intravenous injection or infusion. The immune globulin preparation contains one or more non-ionic surface active agents in a physiologically compatible buffered medium. Inclusion of said non-ionic surface active agent in the preparation surprisingly prolongs the serum half-life of the immune globulin *in vivo* and improves the safety profile of the product. For instance, a preparation with an extended half-life means that the active therapeutic ingredient would have a longer survival time in the bloodstream to exert its desired therapeutic effect. A longer serum survival time would also enable a lower frequency of drug administration thereby resulting in a more convenient dosing schedule with fewer injections to improve patient compliance and to reduce indirect costs associated with parenteral administration of the drug. Moreover, a longer serum survival time of a time may translate into lower maintenance doses required to maintain an effective serum drug concentration thereby minimizing the direct cost of drug therapy.

One aspect of the present invention provides a pharmaceutical preparation comprising a relatively pure and non-aggregated immune globulin suitable for intravenous injection or infusion and one or more non-ionic active surface active agents in a physiologically compatible buffered medium. The preparation possesses the novel characteristic of an extended serum half-life *in vivo* and reduced immunogenicity in comparison to equivalent immune globulin preparations without the non-ionic surface active agent. The preparation

may be in the format of a liquid formulation or may be lyophilized to form a powder formulation. The liquid formulation may be administered directly, while the lyophilized powder format may be reconstituted in a physiologically compatible medium before drug administration.

In an embodiment, the immune globulin in the preparation of the present invention is a human immune globulin prepared by extraction from human plasma using the conventional cold ethanol fractionation method followed by a method to render the preparation suitable for intravenous administration or by chromatographic procedures. Said immune globulin or binding partner may also be a monoclonal antibody or binding partner produced by recombinant DNA or hybridoma technology.

In another embodiment, the immune globulin in the preparation of the present invention is an anti-D (also known as anti-Rh_o or anti-Rh_oD) immune globulin; an anti-C (also known as anti-rh') immune globulin; an anti-E (also known as anti-rh") immune globulin; an anti-c (also known as anti-hr') immune globulin or anti-e (also known as anti-hr") immune globulin. Said immune globulin may be prepared by conventional cold ethanol fraction followed by a method to render the preparation suitable for intravenous administration, by chromatographic techniques or by recombinant DNA/hybridoma technology.

In a further embodiment, the non-ionic surface active agent used in the preparation of the present invention is a sorbitan ester or a polyoxyethylene sorbitan ester of a fatty acid.

Another aspect of the invention provides a method of extending the serum half-life or altering the immunomodulatory effect of an immune globulin comprising the addition of sufficient amount of one or more non-ionic surface active agents to the immune globulin formulation. Said immune globulin may a human immune globulin extracted from plasma using conventional ethanol fractionation followed by a method to render the preparation suitable for intravenous

administration, by chromatographic methods, or it may be a monoclonal antibody or binding partner produced by recombinant DNA or hybridoma technology. More specifically, the immune globulin may be an anti-D (also known as anti-Rh_o or anti-Rh_oD) immune globulin; an anti-C (also known as anti-rh') immune globulin; an anti-E (also known as anti-rh") immune globulin; an anti-c (also known as anti-hr') immune globulin or anti-e (also known as anti-hr") immune globulin.

In a further aspect of the invention, the immune globulin formulation is administered to a mammal by parenteral injection or infusion to elevate the circulating immune globulin levels.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

<u>BRIEF DESCRIPTION OF THE DRAWINGS</u>

The invention will now be described in relation to the drawings in which:

Figure 1 is a graph of mean anti-Rh_oD immune globulin blood levels after an intramuscular injection of WinRho SD™. Mean serum anti-Rh_oD immune globulin results are shown for formulations with and without 0.01% (w/v) Polysorbate 80. The solid diamonds show the results from subjects injected with the conventional formulation of WinRho SD™ in 0.9% (w/v) sodium chloride solution. The shaded boxes show the results in subjects injected with new formulation of WinRho SD™ with Polysorbate 80 in 0.9% (w/v) sodium chloride solution.

<u>DETAILED DESCRIPTION OF THE INVENTION</u>

The following non-limiting examples are illustrative of the present invention:

Examples

Example 1

As hereinbefore mentioned, the present inventors have found that the addition of a non-ionic surface active agent to a preparation of relatively pure and non-aggregated immune globulin suitable for intravenous injection or infusion favourably alters the pharmacokinetics of the immune globulin as a therapeutic agent. The inclusion of said non-ionic surface active agent in the formulation prolongs the survival time or serum half-life of said immune globulin. While not wishing to be bound to a particular theory, the mechanism underlying the pharmacokinetic-altering effect of polyoxyalkylene derivatives may involve a change in the basic physicochemical structure of the immune globulin. An altered tissue distribution of the immune globulin (*e.g.* reduced reticuloendothelial uptake and degradation) may not be a valid explanation as the volume of distribution of the immune globulin in some cases indicate limited tissue accumulation.

The inventors have also found that the addition of a non-ionic surface active agent to a preparation of relatively pure and non-aggregated immune globulin suitable for intravenous injection or infusion reduced the elevation of patient neutrophil counts observed with a conventional immune globulin without said non-ionic surface active agent. While not wishing to be bound to a particular theory, the presence of a non-ionic surface active agent reduces the activation of the cytokine cascade and consequently prevents neutrophil induction.

An example of immune globulin that can be used in the present invention is Rh immune globulin or Rh antibodies. Rh antibodies include anti-D (also known as anti-Rh_o or anti-Rh_oD); anti-C (also known as anti-rh'); anti-E (also known as anti-rh''); anti-c (also known as anti-hr') and anti-e (also known as anti-hr''). The Rh antibodies of the present invention may be preparations from plasma enriched for Rh antibodies, polyclonal antibodies, monoclonal antibodies, antibody fragments (e.g. Fab, and $F(ab')_2$), and those produced by recombinant DNA technology. Other immune globulin preparations

suitable for intravenous injection or infusion, such as varicella zoster immune globulin (Varitect® by Biotest Pharma) or cytomegalovirus immune globulin (Cytogam® by Connaught), can also benefit from the present invention.

Immune Globulin Production

Preparations with a high Rh antibody content suitable for intravenous injection or infusion may be isolated as an immune globulin fraction from plasma, preferably human plasma, using conventional techniques. For example, they may be isolated using: (a) the Cohn cold ethanol fractionation method or modifications thereto (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962); (b) ion-exchange chromatographic methods (*e.g.* using DEAE-Sephadex) and modifications thereto may be used to produce Rh antibodies of higher product yield and quality (Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964); and/or (c) anion-exchange chromatographic method as taught in Canadian Patent No. 1,201,063, and modifications thereto. Commercially available anti-Rh_oD immune globulin preparations may also be used in the methods. For example, anti-Rh_oD preparations such as WinRho® or WinRho SD® (Cangene Corporation) may be used in the present invention.

In an embodiment of the invention, an anti-Rh_oD immune globulin fraction is prepared by contacting an aqueous plasma fraction containing IgG with one or more chromatographic separation columns to produce a purified IgG-rich fraction. The aqueous plasma fraction used in the process may be normal non-immunized plasma from an animal source, preferably a human source, or hyperimmune plasma such as plasma from Rh alloimmunized donors.

For example, the Rh_oD antigen is used to immunize the animal through intramuscular, subcutaneous, intraperitoneal, or

intraocular injection, with or without an adjuvant such as Freund's complete or incomplete adjuvant. With the option of booster immunizations, samples of serum are collected and tested for reactivity to the antigen in standard assays (described below). Particularly preferred polyclonal antisera will give a signal on one of the assays that is at least three times greater than background. Once the titre of the animal has reached a plateau in terms of antigen reactivity, larger quantities of the antisera may be obtained readily either by periodic bleeding or by exsanguinating the animal.

Human anti-Rh_oD immune globulin may also be produced in human volunteers. For example, an anti-Rh_oD immune globulin preparation may be obtained from a subject who is naturally immunized (*e.g.* from an Rh incompatible pregnancy) or artificially immunized using Rh-positive blood cells or Rh_oD antigen.

Anti-Rh_oD immune globulin-containing plasma collected from animal or human is modified to the ionic strength and pH of the initial buffer used with the chromatographic separation column. In an embodiment of the invention, the aqueous animal plasma fraction is contacted with one or more, preferably one to two, anionic exchangers to produce a purified IgG-rich fraction.

By way of example, the aqueous animal or human plasma fraction is applied to an anion exchange column which may contain an agarose cross-linked anionic exchange resin such as DEAE-Sepharose CL6B, TMAE Fractogel or DEAE Sephadex A-50, and an IgG-rich fraction is obtained by eluting with an equilibrating buffer. The IgG-rich fraction may be concentrated for example by ultrafiltration.

The purified IgG protein may optionally be treated with a solvent and detergent to inactivate lipid envelope viruses. Suitable solvents and detergents which may be used include Triton X-100 and tri(n-butyl) phosphate (Horowitz, B., *Curr. Stud. Hematol. Blood Transfus.* 56: 83-96, 1989). After the process, said solvents and detergents may be removed using conventional methods such as reverse phase chromatography.

Monoclonal immune globulins may also be produced readily using recombinant and hybridoma techniques (see Canadian Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody (see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988). It will be apparent to one skilled in the art that the fractionation and recombinant approaches may be applied to diverse types of immune globulins. For example, specific monoclonal immune globulins against different antigens may be generated by techniques based on the same principle of recombinant DNA technology.

Non-Ionic Surface Active Agents

Non-ionic surface active agents of the present invention may be Tween® or Span® type surface active agents.

Span® type agents are partial esters of common fatty acids and sugar alcohol anhydrides derived from sorbitol. Said common fatty acids derived from sorbitol are preferably lauric acid, palmitic acid, stearic acid or oleic acid derived from sorbitol. For example, Span 20 is sorbitan monolaurate, Span 40 is sorbitan monopalmitate, Span 60 is sorbitan monostearate, Span 65 is sorbitan tristearate, Span 80 is sorbitan monooleate, and Span 85 is sorbitan trioleate.

Tween® type agents are derivatives of Span® products with polyoxyethylene chains attached to non-esterified hydroxyl groups. Said common fatty acids derived from sorbitol are preferably lauric acid, palmitic acid, stearic acid or oleic acid derived from sorbitol. For

example, Tween 20 is polyoxyethylene (20) sorbitan monolaurate, Tween 21 is polyoxyethylene (4) sorbitan monolaurate, Tween 40 is polyoxyethylene (20) sorbitan monopalmitate, Tween 60 is polyoxyethylene (20) sorbitan monostearate, Tween 61 is polyoxyethylene (4) sorbitan monostearate, Tween 65 is polyoxyethylene (20) sorbitan tristearate, Tween 80 is polyoxyethylene (20) sorbitan monooleate, Tween 81 is polyoxyethylene (5) sorbitan monooleate, and Tween 85 is polyoxyethylene (20) sorbitan trioleate.

Non-ionic surface agents such as sorbitan esters or polyoxyethylene sorbitan esters of fatty acids may be prepared by methods well known in the art. Said surface active agents may also be obtained commercially from J.T. Baker Inc. (Phillipsburg, New Jersey, USA), ICI Atkemix (Brantford, Ontario, Canada), Van Waters and Rogers Ltd. (Richmond, British Columbia, Canada), or Nikkol Co. (Japan).

Immune Globulin Formulation

Formulation of the anti-Rh_oD immune globulin of the present invention involves the addition of an amount of a non-ionic surface active agent sufficient to extend the serum half-life or to alter the immunomodulatory effect of the immune globulin to the IgG-rich concentrate obtained as described above. The immune globulin preferably is at least about 95% pure, more preferably about 99.5% pure, contains at least 94% monomeric IgG, and has not been subjected to chemical or enzymatic modification. A preferred non-ionic surface active agent is Polysorbate 80 which is added to a final concentration of about 0.01% to about 0.5%. Sodium chloride may be added to the formulation to a final concentration of up to about 0.9%. An additional stabilizing agent such as L-glycine or L-histidine may be added to a final concentration of about 0.025M to 0.05M. A preferred preparation contains: a pharmacologically effective amount of human anti-Rh_oD immune globulin (about 3-8%); sodium chloride at about 0.25% (w/v); no or very low level buffer with essentially no ionic strength; Polysorbate 80 at a concentration of about 0.01%-0.02% (w/v); and L-glycine at a concentration of about 0.1M.

The anti-Rh_oD immune globulin formulation is aseptically filtered again through a 0.22 micron filter and filled into vials or ampoules. Filling operations are conducted under aseptic conditions and the fill volume per vial is calculated so that each vial contains a pharmacologically effective amount of anti-Rh_oD immune globulin. This specific amount or volume can vary depending upon the intended route of administration and therapeutic use. The target filling volume is also calculated with sufficient excess to allow for variation in the potency assay and/or possible loss of potency during storage.

The final aqueous formulation may be lyophilized using a Virtus 251 SRC-5 Sublimator (or equivalent). Lyophilization (also termed vacuum freeze-drying or sublimation) is commonly used in the manufacture of protein pharmaceuticals to improve the stability of the product and extend its shelf life. The lyophilization process is often be divided into three stages: freezing; primary drying (also termed ice sublimation); and secondary drying (also termed water desorption). The starting aqueous solution containing the protein is frozen and the ice is subsequently sublimed thereby leaving a dry porous mass of protein which is stable and can be reconstituted rapidly in water. The technical parameters of the lyophilization process including temperature (eutectic and collapse), vacuum pressure and atmospheric gas composition, are fully automated by the Virtus 251 SRC-5 Sublimator. The basic theories and more practical aspects of protein lyophilization and formulation are described in detail in Skrabanja, A.T. et al. (*J. Pharm. Sci. Technol.* 48: 311-317, 1994); Rey, L.R. (*Dev. Biol. Stand.* 74: 3-8, 1992); Pikal, M.J. (*BioPharm* October, 26-30, 1990); Pikal, M.J. (*J. Parenter. Sci. Technol.* 39: 115-138, 1985); Williams, N.A. and Polli, G.P. (*J. Parenter. Sci. Technol.* 38: 48-59, 1984; Nail, S.L. (*J. Parenter. Drug Assoc.* 34: 358-368, 1980); Ito, K. (*Chem. Pharm. Bull.* 19: 1095-1102, 1971).

In the case of a lyophilized powder formulation, the powder comprising the immune globulin and the non-ionic surface active agent is reconstituted in a physiologically compatible diluent such as sterile water for injection or saline before parenteral administration. For

example, 120 ug (600 IU) or 300 ug (1,500 IU) of a commercial anti-Rh_oD immune globulin product, WinRho SD™, is reconstituted in 2.5 mL diluent.

Therapeutic dosage of immune globulin preparation

Dosages of anti-Rh_oD immune globulin in the formulations of the present invention depend on individual needs, on the protein content/concentration of the immune globulin preparation, on the desired effect in a particular therapeutic indication, and on the chosen route of drug administration. Daily dosages of an anti-Rh_oD immune globulin preparation (3% to 8% wt-solution) for humans by intramuscular or intravenous injection generally vary between about 50 IU (10 ug) to 2,000 IU (400 ug) per kg body weight. For intramuscular injection, the preferred dosage is about 100 IU (20 ug) to 2,000 IU (400 ug) per kg body weight. For intravenous injection, the preferred dosage is about 50 IU (10 ug) to 1,000 IU (200 ug) per kg body weight, preferably 250 IU (50 ug) per kg body weight. The recommended dosage of Biotest Pharma's intravenous varicella zoster immune globulin preparation, Varitect®, is about 50 IU per kg body weight for the shingles therapy and are lower (12 to 25 IU per kg body weight) for chickenpox prophylaxis. In contrast, the recommended dosages of general intravenous immune globulin products (4.5-5.5 wt-% solution) such as Gamimune®, Iveegam® or Sandoglobulin® are significantly higher at about 100 mg to 800 mg per kg body weight.

Pharmacokinetics of anti-Rh_oD immune globulin given as Polysorbate 80 formulation

Methods. The Pharmacokinetics of anti-Rh_oD immune globulin formulations with and without Polysorbate 80 were assessed in a single-centre, randomized, parallel arm study. Twenty-four human subjects (normal, healthy male and female volunteers of age 18 to 55 years) were randomized into two groups to receive 600 µg (3,000 IU) of a commercial brand of anti-Rh_oD immune globulin, WinRho SD®. Twelve subjects received conventional WinRho SD® formulation without Polysorbate 80, and the other 12 subjects received WinRho SD® formulation with

0.01% (w/v) Polysorbate 80. Each formulation was given intramuscularly as two 1.25 mL injections with the test articles being lyophilized human anti-Rh_oD immune globulin in 0.9% (w/v) saline for intravenous injection with or without Polysorbate 80.

Screening assessments were conducted within three weeks of drug administration. Baseline assessments were conducted on the morning of the day that WinRho SD™ was administered to the subjects and before drug administration. These included haematology, blood chemistry and urinalysis. Demographics, vital signs and baseline laboratory tests were compared for study subjects randomized to the different arms of treatment. There was no statistically significant difference between the two treatment groups in any assessments prior to drug administration.

Subjects remained under observation for eight hours and blood samples for anti-Rh_oD immune globulin analysis were drawn from the study subjects to provide 5 mL serum samples at the following times after study drug administration: 8 hours, 24 hours, 3 days, 7 days, 11 days, 14 days, 21 days, and 28 days. Subjects also underwent haematology, blood chemistry and urinalysis laboratory testing at 7 days and 28 days after WinRho SD™ injection. Anti-Rh_oD immune globulin concentration in patient samples is analyzed by conventional techniques (see Auto-Analyzer technique in Moore, B.P.L., *Can. Med. Assoc. J.* 100: 381-387, 1969).

Pharmacokinetics. Regressions were performed on log transformed corrected serum anti-Rh_oD immune globulin levels against time to determine lambda and subsequently, the estimated half life of drug in the study subjects. Blood levels after the day 3 draw were used in these regressions as peak serum levels of anti-Rh_oD immune globulin was usually obtained by day 3 after injection of drug. Significant linear relationships ($p<0.02$) existed between the variables Log (corrected anti-Rh_oD immune globulin) and Time for all study subjects.

The mean time to peak anti-Rh_oD immune globulin levels after intramuscular injection of 600 µg of WinRho SD™ was about 3 days and peak serum anti-Rh_oD immune globulin levels that were achieved were about 70 ng/mL. There was no statistically significant difference between the two formulations and the mean time to peak or peak levels of drug. Similarly, the $AUC_{28\ day}$ was about 1250 days•ng/mL and there was no difference between the two formulations in these values.

The mean half-life was different for the two formulations of WinRho SD™. The formulation of drug without Polysorbate 80 had an mean apparent serum half-life of 16.4 ± 3.8 days, while the mean apparent half-life of WinRho SD™ in the subjects that received formulation of WinRho SD™ with Polysorbate 80 was 20.3 ± 3.4 days. This difference was statistically significant (p=0.012) in a Student t test of the difference in the means. Key pharmacokinetic data are presented in Table 1.

TABLE 1

	Formulation without Polysorbate 80	Formulation with Polysorbate 80	Significance
Time to Peak (days) Mean ± SD Median Range	 2.83 ± 0.14 3.00 1.00 - 3.04	 3.65 ± 1.55 2.99 2.96 - 6.99	p=0.100
Peak Anti-D (ng/mL) Mean ± SD Median Range	 76.2 ± 12.2 79.7 54.76 - 99.09	 68.8 ± 11.8 68.8 49.28 - 88.39	p=0.157
AUC (days•ng/mL) Mean ± SD Median Range	 1225 ± 195 1278 938 - 1564	 1273 ± 268 1174 982 - 1803	p=0.673
Serum Half-Life (days) Mean ± SD Median Range	 16.4 ± 3.6 17.0 9.04 - 21.91	 20.3 ± 3.4 20.6 15.07 - 26.74	P=0.012

Safety. Twenty-four subjects completed 28 days of participation in this study and no subject was withdrawn because of adverse experience. There were a total of 33 adverse events reported in this study (see Table 2). The majority of the events occurred in the Body As A Whole (13 events) and the Respiratory System (9 events). For the most part, the

events were evenly distributed between the two different arms of treatment. An exception were 3 adverse events that occurred in the Digestive System; two reports of dyspepsia and one report of vomiting occurred in subjects receiving old formulation of WinRho SD™. However, since none of the events that occurred in the Digestive System were believed to be related to WinRho SD™ injection, this observation is not considered significant.

TABLE 2

Preferred Term	All Reports		Formulation without Polysorbate 80		Formulation with Polysorbate 80	
	# Events	# (%)	# Events	# (%)	# Events	# (%)
BODY AS A WHOLE	13	13 (54%)	6	6 (50%)	7	7 (58%)
CARDIOVASCULAR SYSTEM	1	1 (4%)	0	0	1	1 (8%)
DIGESTIVE SYSTEM	3	3 (12%)	3	3 (25%)	0	0
NERVOUS SYSTEM	3	3 (12%)	2	2 (17%)	1	1 (8%)
RESPIRATORY SYSTEM	9	6 (25%)	4	3 (25%)	5	3 (25%)
SPECIAL SENSES	2	2 (8%)	0	0	2	1 (17%)
UROGENITAL SYSTEM	2	1 (4%)	2	1 (8%)	0	0

On the morning of study drug administration and before drug injection, a baseline assessment was conducted that included Vital Signs. The Vital Signs were then assessed in study subjects at 1 hour, 3 hour, 8 hour, 24 hour, 7 day and 28 days after drug administration. There were no statistically significant changes in group Vital Signs from baseline and all mean group changes in Vital Signs were within a standard deviation of zero. The largest changes in vital signs relative to the variance was in the temperature of the subjects receiving the old formulation of WinRho SD™ at early times after drug administration. Body temperature was elevated by 0.3±0.4 °C at 1 hour, 0.3±0.3 °C at 3 hours and 0.5±0.5 °C at 8 hours after WinRho SD™ injection as compared to increases in this group of 0.2±0.4 °C at 24 hours, 0.1±0.6 °C at 7 days and 0.1±0.4 °C at 28 days after drug administration. In contrast, the study subjects who received the new formulation of WinRho SD™ had body temperature increases of 0.0±0.5 °C, 0.1±0.5 °C, 0.2±0.5 °C, 0.0±0.4 °C, 0.1±0.7 °C and

0.1±0.4 °C at the assessments after drug administration. Given the low statistical significance to these changes in body temperature, it is not clear if they are related to WinRho SD™ administration. If they are, then the changes are subclinical and the pyrogenic effect is smaller with the new formulation of WinRho SD™.

On the morning of study drug administration and before drug injection, a baseline assessment was conducted that included Laboratory testing. These laboratory tests were repeated in study subjects at 7 days and 28 days after injection of WinRho SD™. Day 7 laboratory data was not significantly different from baseline data and mean Δparameter values were within a standard deviation of zero. However, the mean neutrophil count for the patients receiving old formulation was 4.67% ± 4.72% seven days after drug administration and this change was statistically different (p=0.040) from the mean increase of -0.75% ± 7.20% in neutrophils 7 days after administration of the new formulation of WinRho SD™. This difference accounted, in part, for the differences between arms in the proportion of WBC that were neutrophils (61% *vs.* 53%) and in the bands ($4.44x10^9$/L *vs.* $3.07x10^9$/L) at 7 days after drug administration.

There are no statistically significant differences in treatment arms in the Haematology laboratory data obtained 28 days after drug administration. There was an overall decrease of 1.3% in the Haematocrit of the study subjects 28 days after WinRho SD™ that may have been related to the frequent phlebotomy associated with participation in the study.

There were differences in the 7 day ΔALT and ΔBilirubin with data from the subjects receiving new formulation being closer to baseline values than data from subjects receiving old formulation. However, the mean differences reflected subclinical changes and there were no differences in mean ALT and mean Bilirubin data for these groups. As such, these differences were believed to be fortuitous and result from the frequent hypothesis testing in this analysis. There were no statistically

significant differences between the treatment arms in the Clinical Chemistry data obtained 28 days after study drug administration.

Conclusion. The new formulation of WinRho SD™ that contains Polysorbate 80 had the same, or a better, safety profile as the currently licensed WinRho SD™. This is consistent with the relatively high LD50 values for non-ionic surface active agents in mammals (*supra*). This new formulation also improved the appearance and stability of WinRho SD™ when reconstituted. Surprisingly, the new formulation with Polysorbate 80 has a longer half life (20.3 days) than the old formulation (16.4 days). This difference is beneficial in the therapeutic use of the drug. For example, the new WinRho SD™ formulation with Polysorbate 80 would lead to higher passive anti-Rh_oD immune globulin levels at long times after drug administration in prophylaxis of Rh Immunization of Rh negative patients. Moreover, inclusion of Polysorbate 80 in the anti-Rh_oD immune globulin preparation significantly minimized drug-induced elevations of neutrophil counts in the recipients and altered the immunomodulatory effect of the immune globulin.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

DETAILED FIGURE LEGENDS

Figure 1 is a graph of mean anti-Rh_0D immune globulin blood levels after an intramuscular injection of WinRho SD™. Mean serum anti-Rh_0D immune globulin results are shown for formulations with and without 0.01% (w/v) Polysorbate 80. The solid diamonds show the results from subjects injected with the conventional formulation of WinRho SD™ in 0.9% (w/v) sodium chloride solution. The shaded boxes show the results in subjects injected with new formulation of WinRho SD™ with Polysorbate 80 in 0.9% (w/v) sodium chloride solution.

We Claim:

1. An aqueous immune globulin preparation comprising an immune globulin and a non-ionic surface active agent in a concentration sufficient to increase the serum half-life of the immune globulin.

2. The preparation according to claim 1 wherein the immune globulin is anti-Rh_oD immune globulin with an IgG purity of greater than 95% and a monomeric protein content of greater than 94%.

3. The preparation according to claim 1 wherein the immune globulin is anti-c immune globulin with an IgG purity of greater than 95% and a monomeric protein content of greater than 94%.

4. The preparation according to claim 1 wherein the concentration of the immune globulin is about 2 weight percent to about 10 weight percent.

5. The preparation according to claim 1 wherein the non-ionic surface active agent is a sorbitan ester of a fatty acid.

6. The preparation according to claim 5 wherein the non-ionic surface active agent is selected from the group consisting of sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan tristearate, sorbitan monooleate, and sorbitan trioleate

7. The preparation according to claim 1 wherein the non-ionic surface active agent is a polyoxyethylene sorbitan ester of a fatty acid.

8. The preparation according to claim 7 wherein the non-ionic surface active agent is selected from the group consisting of polyoxyethylene (20) sorbitan monolaurate; polyoxyethylene (4) sorbitan monolaurate; polyoxyethylene (20) sorbitan monopalmitate;

polyoxyethylene (20) sorbitan monostearate; polyoxyethylene (4) sorbitan monostearate; polyoxyethylene (20) sorbitan tristearate; polyoxyethylene (20) sorbitan monooleate; polyoxyethylene (5) sorbitan monooleate; and polyoxyethylene (20) sorbitan trioleate.

9. The preparation according to claim 1 wherein the concentration of the non-ionic surface active agent is about 0.01 weight percent to about 0.5 weight percent.

10. The preparation according to claim 1 wherein the immune globulin preparation is lyophilized to form a dry powder preparation.

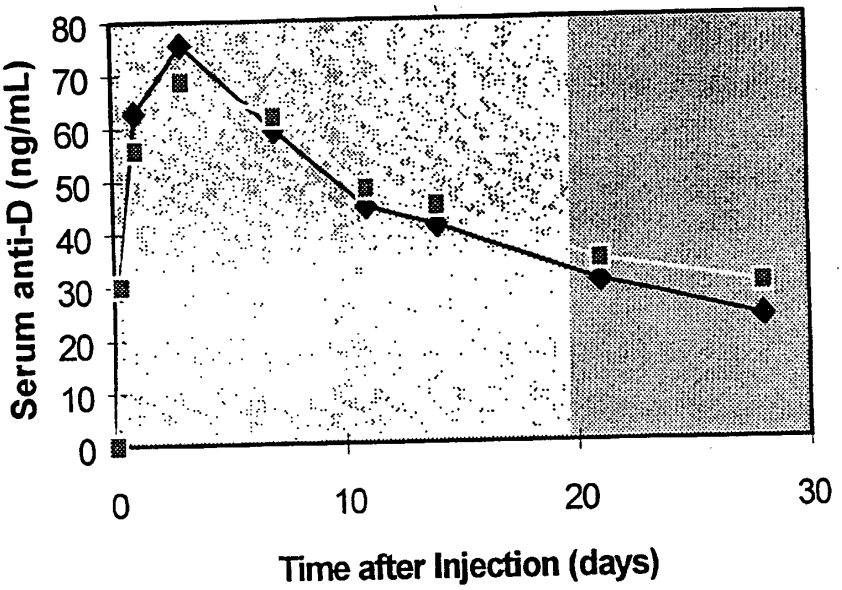
11. A method of increasing the serum half-life of an immune globulin preparation comprising administering an immune globulin preparation according to claims 1 to 10 to a patient in need of such a treatment.

12. A method according to claim 11 wherein said immune globulin preparation is administered intravenously.

ABSTRACT OF THE DISCLOSURE

Addition of a non-ionic surface active agent to an immune globulin formulation extends the serum half-life of relatively pure and non-aggregated immune globulin suitable for intravenous injection or infusion. The non-ionic surface active agent may be a sorbitan ester or a polyoxyethylene sorbitan ester of a fatty acid. Formulations of the present invention is therapeutically advantageous over conventional formulations in that an extended serum half-life of the immune globulin improves its therapeutic effectiveness, reduces the frequency of drug administration and/or lowers the therapeutic effective dosage required and cost of treatment.

FIGURE 1


Serum anti-D (ng/mL)
80
70
60
50
40
30
20
10
0
0
10
20
30
Time after Injection (days)